Appl. No. 09/442,111
Amdt. dated April 12, 2007
Amendment under 37 CFR 1.116 Expedited Procedure
Examining Group 1652

REMARKS/ARGUMENTS

With this amendment, claims 53, 55-58 and 60-74 are pending. Claims 1-52, 54, and 58-59 are cancelled. For convenience, the Examiner's rejections are addressed in the order presented in an October 13, 2006 Office Action.

I. Status of the claims

Claim 53 is amended to recite permeabilizing a host cell that comprises a heterologous accessory enzyme and a heterologous accessory enzyme. Support for this amendment is found throughout the specification, for example, at page 44, lines 7-14 and original claims 57. The permeabilized host cell is the contacted with an exogenous acceptor saccharide. Support for this amendment is found throughout the specification, for example, at page 7, lines 26-29 and at page 44, lines 7-9. Claim 57 is amended to recited use of 1% Xylene to permeabilize a cell. Support for this amendment is found throughout the specification, for example, at page 50, line 28 through page 60, line 2. Claim 71 is amended to recite that the heterologous accessory enzyme is a CMP-sialic acid synthetase. Support for this amendment is found throughout the specification, for example, at page 60, lines 20-29. These amendments add no new matter.

II. Rejections under 35 U.S.C. §112, first paragraph, written description

Claims 53, 55-58 and 60-74 are rejected under 35 U.S.C. §112, first paragraph for allegedly failing to comply with the written description requirement. According to the Office Action, the specification lacks description of the claimed invention, such that a skilled artisan would recognize that Applicants had possession of the claimed invention at the time of filing. In particular, the Office Action appears to assert that the genus of glycosyltransferases, accessory enzymes, and oligosaccharides are not adequately described because, allegedly, structural features of the genera are not disclosed. Applicants respectfully traverse the rejection. The Examiner's position is incompatible with recent Federal Circuit rulings on the written description requirement.

The claims are directed to cell-based methods of producing product saccharides by expressing a heterologous accessory enzyme for forming a nucleotide sugar and a heterologous glycosyltransferase in the same cell; permeabilizing the cell; allowing formation of the nucleotide sugar through the activity of a heterologous accessory enzyme; and contacting the cell with an exogenous acceptor saccharide to allow transfer of the sugar to the acceptor saccharide through the activity of the heterologous glycosyltransferase. Many glycosyltransferases and accessory enzyme sequences were known at the time of filing and the specification provides detailed information, including accession numbers, to assist those of skill in accessing the sequence information. Applicants have pointed to the support for the known sequence information in more than one previous response and direct the Examiner to those responses for detailed information. In addition, Applicants also assert again that names of oligosaccharides; *e.g.*, 3' sialyllactose, β1,4-GalNAc-lactose, and the formulas listed in Table 3; do allow those of skill to immediately envision the structure of the oligosaccharides.

Applicants also respectfully assert that the specification provides written description of glycosyltransferase and accessory enzyme sequences known at the time of filing. Therefore, as the discussion of Federal Circuit case law below makes clear, the specification as filed meets the written description requirement. The Federal Circuit Court of Appeals has made it clear that there is no *per se* rule regarding inclusion of sequence information in a patent application to support description of a nucleic acid sequence, and by analogy an amino acid sequence. "When the prior art includes the nucleotide information, precedent does not set a per se rule that the information must be determined afresh." *Capon v. Eshar*, 76 USPQ2d 1078, 1084-5 (Fed. Cir. 2005). In fact, the Federal Circuit has recently ruled that even incorporation by reference of known sequences is not required for the written description requirement. "Accordingly we hold that where, as in this case, accessible literature sources clearly provided, as of the relevant date, genes and their nucleotide sequences. . . , satisfaction of the written description requirement does not require either the recitation or incorporation by reference (where permitted)of such genes and sequences." *Falkner v. Inglis*, 79 USPQ2d 1001, 1008 (Fed. Cir. 2006). In *Falkner* the court also stated that ". . . [A] requirement that patentees recite known

DNA structures, if one existed, would serve no goal of the written description requirement." *Falkner*, 79 USPQ2d at 1009. Thus, under the rulings of *Capon* and *Falkner*, the application meets the written description requirement for the claimed methods.

Although Applicants do not to include them with this response, arguments against the rejection for alleged lack of written description asserted in previous responses are maintained.

Based on the disclosure of the specification, one of skill would be able to practice the claimed methods. The Office Action's insistence that the sequence of accessory enzymes and glycosyltransferases must be disclosed in the specification to meet the description requirement is not appropriate under current Federal Circuit case law.

In view of the above arguments, withdrawal of the rejections for alleged lack of written description is respectfully requested.

III. Rejections under 35 U.S.C. §103(a)

The Office Action maintains rejections for alleged obviousness in view of various combinations of references. To the extent the rejection apply to the amended claims, Applicants respectfully traverse the rejections.

The Office Action has not established a case of *prima facie* obviousness. To establish a case of *prima facie* obviousness, the Examiner must meet three basic criteria:

> First, there must be some suggestion or motivation, either in the references themselves or in the knowledge generally available to one of ordinary skill in the art, to modify the references or to combine reference teachings. Second, there must be a reasonable expectation of success. Finally, the prior art reference (or references when combined) must teach or suggest all the claim limitations. *In re Vaeck*, 947 F.2d 488, 20 USPQ2d 1438 (Fed. Cir. 1991). M.P.E.P. §§ 706.02(j) and 2143.

The references cited by the Examiner fail to provide a reasonable expectation of success in practicing the invention and fail to provide a motivation for the combination of the

references. In addition, the references cited by the Examiner fail to provide all the elements of the rejected claims. Therefore, Applicants respectfully traverse the rejections.

Claims 53, 56-58, 60, and 65-74 are rejected as allegedly obvious over Samain *et al.*, *Carbohy. Res.* 302:35-42 (1997) in view of Ullrich and van Putten, *J. Bacteriol.* 177:6902-6909 (1995). Applicants respectfully traverse the rejection. The amended claims are directed to a cell based method of producing an oligosaccharide, by using a host cell that comprises a heterologous glycosyltransferase and a heterologous accessory enzyme to produce a sugar nucleotide. The host cells are permeabilized and than contacted with an exogenous acceptor saccharide. The nucleotide sugar is produced through the activity of the heterologous accessory enzyme and then the sugar moiety is transferred from the nucleotide sugar to the exogenous acceptor saccharide through the activity of the heterologous glycosyltransferase. The cited references, either alone or combined, fail to provide all the elements of the claimed invention, fail to provide motivation for combination of the references, and fail to provide a reasonable expectation of success in arriving at the claimed invention.

Samain *et al.* relies on intracellular acceptor substrates, not exogenous acceptor substrates as recited in the amended claims. Instead, Samain *et al.* disclose use of intracellularly produced GlcNAc or GlcNAc polymers as the acceptor substrate. Samain *et al.* provide no teaching of an exogenous substrate and further, provide no suggestion that any substrate not produced inside the cell would be necessary to produce CO's in *E. coli*.

Samain *et al.* do not permeabilize cells before production of an oligosaccharide product, rather, Samain *et al.* disrupt cells with extremely harsh methods after production of COs has ended. Samain *et al.* recover CO's from cells with, *e.g.*, 45 minutes in an autoclave. *See,* Samain *et al.* at page 41, left column. In contrast, because the acceptor substrate is incubated with the permeabilized cells containing, *e.g.*, the heterologous glycosyltransferase, the cell permeabilization of the claimed methods must be gentle enough to maintain enzymatic activity of the glycosyltransferase. *See, e.g.*, specification at page 44, lines 7-14. Any cell disruption disclosed by Samain *et al.* occurs after the chitin product is synthesized, negating any need to

contact a disrupted cell with an acceptor substrate. Thus, the permeabilized cells used in the claimed methods cannot be obtained using the disruption techniques disclosed in Samain *et al.*

Samain *et al.* teach away from use of a heterologous accessory enzyme. Samain *et al.* discloses synthesis of chitooligosaccharides (COs) in *E. coli* by expression of the *A. caulinodans NodC* gene, which encodes a CO synthase. COs are polymers of GlcNAc residues. Samain *et al.* does not disclose or suggest co-expression of a **heterologous** accessory enzyme for enzymatic synthesis of UDP-GlcNAc, the sugar-nucleotide substrate of the NodC protein. In fact, Samain *et al.* teach away from use of anything other than the endogenous *E. coli* sugar nucleotide pools maintained by endogenous enzymes, stating at page 36, left column that UDP-GlcNAc is maintained at "quite high" intracellular levels in most bacteria, thereby providing ample substrate for the NodC protein. Samain *et al.* also cautions that over expression of recombinant gene products can be unfavorable to oligosaccharide synthesis by causing "unnecessary metabolic burning, a growth inhibition by overproduced proteins, and problems of precipitation and misfolding of proteins." Samain *et al.* page 39, left column. Samain *et al.* warns those of skill against over expression of proteins generally, including over expression of multiple proteins and thus, does not provide a reasonable expectation of success or a motivation to combine to express the GlcNAc-1-phosphate uridyltransferase (*GlmU*) gene of Ullrich and va Patton, as suggested by the Office Action.

Ullrich and van Putten does not remedy the deficiencies of Samin *et al.* Ullrich and van Putten discloses cloning of GlcNAc-1-phosphate uridyltransferase (*GlmU*) gene from gonococcus. Ullrich and van Putten does not disclose or suggest co-expression of a glycosyltransferase for enzymatic synthesis of an oligosaccharide. Ullrich and van Putten does not discuss or investigate the affect of *GlmU* expression on levels of either UDP-GlcNAc or oligosaccharides containing GlcNAc. Given Samain *et al.*'s disclosure that UDP-GlcNAc levels are quite high in most cells, one of skill would have no reason to suppose that expression of *GlmU* would have any effect on cellular UDP-GlcNAc levels and therefore would not have reason to attempt to co-express, *e.g.*, NodC and GlmU in the same cell.

Thus, alone or in combination, Samain *et al.* and Ullrich and van Putten provide no suggestion or motivation to a) use an exogenous acceptor molecule or b) permeabilize a cell to allow contact of an exogenous acceptor saccharide with a heterologous glycosyltransferase expressed by the host cell, or c) use a heterologous accessory enzyme. Therefore, the combination of Samain *et al.* and Ullrich and van Putten does not render the claims obvious.

Claims 61-65 are rejected as allegedly obvious over Samain *et al.* in view of Ullrich and van Putten, and in further view of Bulow *et al. Trends Biotech.* 9:226-231 (1991). Applicants respectfully traverse the rejection. Claims 61-65 depend from claim 53. Therefore, the above discussion of Samain *et al.* and Ullrich and van Putten applies to this rejection, but will not be repeated here. This analysis discusses the citation of Bulow *et al.*, but does not repeat arguments submitted with the previous response. Applicants continue to assert the previously submitted arguments, however. Bulow *et al.* disclose generally construction of bi-functional proteins by fusing proteins with different functions together. Bulow *et al.* does not discuss or suggest fusion proteins that include a glycosyltransferase and an accessory enzyme. Bulow *et al.* does not disclose or suggest any type of oligosaccharide synethesis. Finally, Bulow *et al.* does disclose any cell based system for production of any macromolecule.

Bulow *et al.* does not remedy the deficiencies of Samin *et al.* and Ullrich and van Putten and provides no motivation for those of skill to practice the claimed invention. Thus, alone or in combination, Samain *et al.*, Ullrich and van Putten, and Bulow *et al.* provide no suggestion or motivation to a) use an exogenous acceptor molecule or b) permeabilize a cell to allow contact of an exogenous acceptor saccharide with a heterologous glycosyltransferase expressed by the host cell, or c) use a heterologous accessory enzyme. Therefore, the combination of Samain *et al.*, Ullrich and van Putten, and Bulow *et al.* does not render the claims obvious.

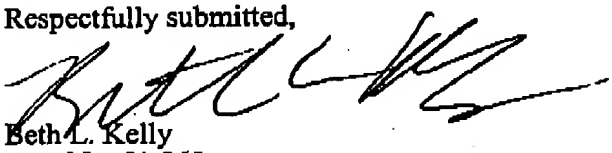
In view of the evidence submitted with this response and the arguments above, Applicants respectfully request that the rejection for alleged obviousness be withdrawn.

CONCLUSION

In view of the foregoing, Applicants believe all claims now pending in this Application are in condition for allowance and an action to that end is respectfully requested.

If the Examiner believes a telephone conference would expedite prosecution of this application, please telephone the undersigned at 415-576-0200.

Respectfully submitted,



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